Changes in Leucocyte Sodium Transport in Normotensive Relatives of Hypertensive Subjects
Dissociation from Blood Pressure


SUMMARY It has been postulated that depressed membrane sodium transport is a necessary step in blood pressure elevation in essential hypertension. Accordingly, leucocyte sodium efflux-rate constants were estimated in 14 normotensive subjects who had one or more first-degree relatives with essential hypertension, and also in 14 matched control subjects with no such family history, before and after taking bendrofluazide for 7 days. Efflux rates in the controls did not change after the diuretic. However, in the relatives, mean total sodium efflux-rate constant was at first significantly depressed but later rose to normal with the diuretic. This was due almost entirely to an increase in glycoside-sensitive sodium pump activity. Blood pressure remained unchanged in both groups. Thus, assuming that perturbations in leucocytes reflect similar abnormalities in other cell lines, major changes in sodium transport in the normotensive individual without accompanying changes in blood pressure suggest that, while these changes may be a marker for later hypertension, they do not participate directly in blood pressure control. (Hypertension 6: 369-373, 1984)

KEY WORDS • leucocyte sodium transport • diuretics • hypertension

The demonstration that the arterial wall of hypertensive patients contained increased sodium and water suggested that elevated blood pressure might be the result of a global abnormality of electrolyte handling; accordingly, interest has been focused on more accessible human blood cells. The leucocyte is a nucleated cell that has a large number of membrane Na⁺,K⁺-ATPase pump sites, and if defective sodium transport is implicated in causing essential hypertension, this cell provides a readily obtainable model to study. While the leucocyte cell does not contribute to increased peripheral resistance, it is possible that it reflects changes in electrolyte transport by vascular smooth muscle. Therefore, abnormalities in leucocytes could provide a valuable marker for an underlying vascular abnormality.

A reduction in glycoside-sensitive sodium pump activity has been demonstrated in the leucocytes of subjects with essential hypertension together with a raised intracellular sodium content. According to one hypothesis, these abnormalities are due to an inherited inability of the kidney to excrete a sodium load, and this inability leads to release of a humoral factor that inhibits Na⁺,K⁺-ATPase. The resulting increase in intracellular sodium inhibits a postulated sodium-calcium exchange process and produces hyperresponsiveness of vascular smooth muscle. It has been further argued that the fall in blood pressure provoked by thiazide diuretics is associated with restoration of depressed sodium pump activity and the return of intracellular sodium to normal.

We have recently demonstrated similar abnormalities of sodium transport in the leucocytes of normotensive first-degree relatives of patients with essential hypertension. We have argued that if such changes reflect electrolyte changes in vascular smooth muscle cells, the abnormalities in sodium transport do not participate directly in blood pressure elevation. However, comparisons between different populations can only yield limited information. Accordingly, to ascertain the significance of abnormal leucocyte electrolyte transport, we studied the effects of diuretic-induced alterations in leucocyte sodium handling on the accompanying blood pressure in normotensive subjects.

Subjects and Methods

Fourteen healthy normotensive subjects were studied, all with one or more first-degree relatives known to have essential hypertension. Nine were men, five
were women, and their mean age was 28 years. These subjects were compared with 14 normotensive controls with no family history of hypertension. Participants were Caucasian except for one Asian subject in each group; they did not differ significantly in mean age, height, or weight, plasma renin activity (PRA), or blood pressure (Tables 1 and 2). Classification of the volunteers was achieved by an initial interview in which each subject documented the blood pressure status of all first-degree relatives. If there was doubt, relatives were asked to have their blood pressure checked at the Department of Medicine or by their family doctor. If doubt still remained, the volunteer was not studied. All blood pressures were measured with a Hawksley random-zero sphygmomanometer with supine and upright. Full ethical committee approval was granted, and informed consent was obtained from all volunteers.

### Measurement of Sodium Efflux-Rate Constant

On the day of study, 50 ml of blood was taken by puncture of an antecubital vein with a 20-gauge multipurpose needle (Becton Dickinson, Rutherford, New Jersey) connected to a vacutainer tube containing lithium heparin as an anticoagulant (Becton Dickinson, Rutherford, New Jersey). The blood was transferred to four universal containers holding 7.5 ml of plasmagel (Uniscience, Cambridge, England) and thoroughly mixed. The containers were allowed to stand in a waterbath at 37°C, which facilitated the sedimentation of erythrocytes and the suspension of leucocytes. After 30 minutes, the supernatant was transferred to plastic 10 ml centrifuge tubes (Sarstedt, West Germany) and centrifuged at 37°C at 300 g for 7 minutes. This formed a cell plug at the bottom of the tube that contained leucocytes and residual erythrocytes. The supernatant was removed, and the remaining red cells were destroyed by hypotonic lysis, which was achieved by adding 2 ml of water and then, 13 seconds later, by adding 2 ml of a × 2 Earle's solution, thereby suspending the cells in a × 1 concentration of Earle's buffer. The number of leucocytes obtained for study in this manner was approximately 50% per milliliter of that observed in unprocessed venous blood.

The percentage of white cell types was calculated by microscopic counting of cells per high power field and comprised 70% neutrophils, 18% lymphocytes, 3% eosinophils and basophils combined, and 9% smear cells. Viability of the cells was tested with the Trypan blue exclusion method and found to be 95%. The cell suspension was centrifuged at 37°C at 300 g for 5 minutes, and formed a pellet that now contained leucocytes and just a little red cell debris. The pellet was resuspended in 8 ml of tissue culture medium 199 (Gibco Ltd., Paisley, Scotland) and was ready for study.

A 3 ml aliquot of the cell suspension was taken for subsequent estimation of intracellular electrolytes. The remaining 5 ml was labeled with 5 μCi of 32Na (Radiochemicals, Amersham, England) and incubated at 37°C for 30 minutes to reach a steady state. At the end of this time, the cells were centrifuged for 3 minutes at 37°C at 300 g and resuspended in 6 ml of unlabeled M199 to remove excess isotope from the cells. This procedure was repeated, after which the cell suspension was split into two aliquots of 3 ml each. To one of these was added 0.1 ml of 10⁻³ M ouabain, and samples were taken from both aliquots at regular intervals over 20 minutes. Each pair of samples was spun at 2000 g to stop sodium efflux and precipitate the cells. The supernatant was removed, the tube was dried with paper tissues, and residual radioactivity was counted. The total sodium efflux-rate constant was calculated from the slope of the linear regression curve in the absence of ouabain. Glycoside-sensitive activity (sodium pump activity) was derived by subtracting the rate constant obtained in the presence of ouabain from the total.

### Measurement of Leucocyte Sodium and Potassium

The 3 ml unlabeled aliquot obtained above was allowed to stand at 37°C for 25 minutes. At the end of this time the cells were centrifuged at 0°C and 300 g for 3 minutes and then resuspended in 3 ml of ice-cold magnesium chloride (99 mmol). The cells were again centrifuged at 0°C and 300 g for a further 3 minutes and then resuspended in 1 ml of MgCl₂. The suspension was transferred to a preweighed polythene tube and centrifuged at 2000 g for 5 minutes at 0°C. The supernatant was removed, and the tube was dried and placed in an oven at 100°C for 12 hours to ash the cells. The tube was reweighed, and the ash was placed in deionized water to leach out the sodium and potassium. The sodium and potassium were estimated by flame photometry and the results expressed as mmol per kg of dry weight of cells.
TABLE 3. Leucocyte Sodium Efflux-Rate Constants Before and After Therapy

<table>
<thead>
<tr>
<th>Group</th>
<th>Total</th>
<th>Ouabain-insensitive</th>
<th>Ouabain-sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>2.48±0.07</td>
<td>0.89±0.06</td>
<td>1.59±0.06</td>
</tr>
<tr>
<td>Relatives</td>
<td>1.98±0.06</td>
<td>0.71±0.06</td>
<td>1.26±0.1</td>
</tr>
</tbody>
</table>

Values are means ± SEM. NS = not significant.

In addition to obtaining blood for efflux-rate constant and electrolyte estimations, additional blood was taken for determination of PRA, serum electrolytes, urea, creatinine, and urate. Subjects were then given bendrofluazide 5 mg orally once daily starting on the day after the first blood sample. After 7 days a second blood sample was obtained. Subjects with and without a family history of hypertension were started in random order.

Statistical analysis of efflux-rate constants for the two populations was by Mann-Whitney U test for unpaired observations and sign test with binomial distribution for paired observations. Other comparisons were by paired and unpaired Student’s t test as appropriate.

Results

Sodium Efflux-Rate Constant

Before Diuretic

There was a highly significant reduction in the mean total efflux-rate constant for sodium in the relatives compared to control subjects (p < 0.001). This was due to a significant depression in the ouabain-sensitive component (p < 0.02); the ouabain-insensitive component was not significantly different (p > 0.1) (Table 3).

After Diuretic

There was no significant change in the mean total sodium efflux-rate constant after diuretic administration in the control group. Neither ouabain-insensitive nor ouabain-sensitive efflux was altered (Figure 1). In the relatives, however, there was a highly significant rise in the total sodium efflux-rate constant after administration of the diuretic when compared to before. This was attributable to a rise in the glycoside-sensitive sodium efflux-rate constant; the ouabain-insensitive constant rose slightly but did not achieve statistical significance (p > 0.05) (Figure 2). The effect of the diuretic was to bring the efflux-rate constants for the relatives into the normal range, so that after therapy there was no significant difference between the two groups (Table 3).

Intracellular Sodium Content and Sodium Efflux Rate

Intracellular sodium content was not significantly different in the two groups before therapy (49 ± 5.6 mmol/kg dry weight for the relatives group vs 48 ± 3.6 mmol/kg dry weight for the control group) and did not alter significantly after 1 week of diuretic therapy (51 ± 3.4 vs 50 ± 4.6 mmol per kg dry weight). Sodium efflux rate (the product of intracellular sodium content and total efflux-rate constant) remained unchanged in the normal subjects before and after therapy (120 ± 8.7 vs 118 ± 13.4 mmol per kg h⁻¹). In the relatives, the efflux rate rose significantly after diuretic therapy (93 ± 10.7 vs 125 ± 10.1 mmol per kg h⁻¹, p < 0.02).

Plasma Renin Activity

There was no difference in PRA in relatives compared to control subjects before the diuretic (7.3 ± 0.8 vs 6.3 ± 0.7 ng angiotensin I ml⁻¹ hr⁻¹, p > 0.2). After 7 days of taking bendrofluazide, both the relatives and control subjects showed a marked similar rise in PRA.
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Total Ouabain
Insensitive
Ouabain
Sensitive

FIGURE 2. Leucocyte sodium efflux rate constants (hr⁻¹) in relatives of hypertensive subjects before (O) and after (△) diuretic.

which indirectly indicated good compliance (12.4 ± 1.4 vs 14.6 ± 1.9 angiotensin 1 ml⁻¹ hr⁻¹, p > 0.2).

Serum Electrolytes, Urea, Creatinine and Urate

Serum sodium did not differ significantly in either group before or after the diuretic (Table 4). Serum potassium was similar in both groups before the diuretic, but after 7 days of therapy, it fell significantly in the control group (p < 0.005). In the relatives group, K⁺ also fell after the diuretic, but the value just failed to achieve statistical significance (p = 0.07). Serum urea and creatinine were not significantly different in the groups before or after the diuretic. The urate levels, which were similar before the administration of bendrofluazide, rose significantly in both of the groups (p < 0.05).

Blood Pressure

There was no significant difference in the mean blood pressure of the two groups before the diuretic, nor did it fall significantly in either group after the diuretic (Table 2). Only the change in supine systolic blood pressure in relatives reached statistical significance (p < 0.05). There was no significant difference in the change in either systolic or diastolic blood pressure after diuretic treatment in the two groups.

Discussion

These results confirm a significant reduction in the mean leucocyte sodium efflux-rate constant in normotensive first-degree relatives of patients with essential hypertension when compared to matched control subjects. The reduction was almost solely due to depressed ouabain-sensitive Na⁺,K⁺-ATPase-mediated pump activity and equivalent in degree to that observed in patients with essential hypertension. The reduction was abolished by the administration of a thiazide diuretic for 7 days, without a significant fall in mean blood pressure. The diuretic produced the expected changes in serum electrolytes, serum urea, and PRA, but these values were not significantly different between the relatives and control subjects either before or after the drug. The present study, therefore, points to an inherited abnormality of sodium handling in leucocytes, which is, however, susceptible to environmental influences. Although the nature of both perturbations remains obscure, the results indicate that the abnormality can be dissociated from elevated blood pressure in normotensive relatives of hypertensive patients and can be corrected by diuretics without any change in mean blood pressure. These results therefore suggest that, over the period of our study at least, such an abnormality if present in smooth muscle played no role in blood pressure control. There was no significant difference between the mean blood pressure in either group before or after the diuretic. Although systolic pressure fell in both groups, only supine systolic pressure in relatives reached statistical significance. Diastolic pressure actually rose. It is unlikely that this change is attributable to the dramatic rise in sodium pump activity, for no other component of the relatives' blood pressure reached significance after the diuretic. Furthermore, mean systolic blood pressure was decreased in the control group after therapy, although it

<table>
<thead>
<tr>
<th>Serum concentration (mmol/liter)</th>
<th>Controls Before</th>
<th>After</th>
<th>Relatives Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>140 ± 0.3</td>
<td>140 ± 0.4</td>
<td>140 ± 0.5</td>
<td>140 ± 0.5</td>
</tr>
<tr>
<td>Potassium (mmol/liter)</td>
<td>4.1 ± 0.08</td>
<td>3.83 ± 0.08*</td>
<td>4.1 ± 0.06</td>
<td>3.9 ± 0.09</td>
</tr>
<tr>
<td>Urea (mmol/liter)</td>
<td>4.2 ± 0.3</td>
<td>4.7 ± 0.35</td>
<td>4.8 ± 0.4</td>
<td>5.0 ± 0.2</td>
</tr>
<tr>
<td>Creatinine (μmol/liter)</td>
<td>89 ± 4.5</td>
<td>88 ± 3.9</td>
<td>80 ± 4.0</td>
<td>83 ± 3.8</td>
</tr>
<tr>
<td>Urate (μmol/liter)</td>
<td>353 ± 22</td>
<td>393 ± 21*</td>
<td>369 ± 25</td>
<td>409 ± 30*</td>
</tr>
</tbody>
</table>

Values are means ± SEM.
*p < 0.05 compared to before bendrofluazide.
did not reach statistical significance. But in this group, sodium pump activity actually fell after drug. It therefore appears unlikely that the sodium pump affects blood pressure changes acutely.

It could be argued that alterations in sodium pump activity only have a long-term effect upon blood pressure. However, no such delayed effect can be postulated, as the effects of sodium loading and depletion upon blood pressure in clinical and experimental models of hypertension in the original hypothesis were observed in hours or days.

The failure to show any difference in intracellular sodium concentration despite alterations in the rate constant deserves comment: either other transport processes underwent compensatory adaptation, or, as seems more likely, the method for measuring intracellular sodium was not sensitive enough. According to one hypothesis, changes in intracellular sodium concentration of as little as 5% can modify vascular contractility. Such changes are well within the limits of error of published methods. The reported ranges of leucocyte sodium concentration are very wide, and even with use of atomic absorption the coefficient of variation is 30%.

It seems likely, therefore, that the differences in leucocyte sodium efflux rate in these normotensive groups reflect inherited differences in cell membrane function between the two populations. Thus, in addition to changes in red cell sodium handling, differences in membrane viscosity, phospholipid composition, and calcium binding have been described. The present study does not differentiate between such composition changes in cell membrane structure or changes in circulating factors induced by diuretic treatment. The thiazide diuretic may have inhibited the release of ouabain-like material or may have modified cell membrane lipid composition and fluidity. Our study, however, indicates that within its time scale, the induced changes play no substantial role in blood pressure control. The abnormality in normotensive relatives may serve as a marker for processes that later cause high pressure. In about half of the relatives, the sodium efflux-rate constant was below the range of that in nonrelatives, and it is possible that these relatives have a genetic predisposition to hypertension. Only long-term follow-up studies can confirm or deny such speculation.

Acknowledgments

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References

5. De Wardener HE, MacGregor GA. Dahl’s hypothesis that a saluretic substance may be responsible for a sustained rise in arterial pressure. Its possible role in essential hypertension. Kidney Int 1980;18:1-9
Changes in leucocyte sodium transport in normotensive relatives of hypertensive subjects. Dissociation from blood pressure.
M Milner, A M Heagerty, R F Bing, H Thurston and J D Swales

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